Declaration of Richard Hess

I, Dr. Richard Hess, declare as follows:

1. I am currently a Principal Engineer with Cargill's Health and Nutrition business unit at Cargill. I have held this position for 10 years. I hold a B.S. in Chemical Engineering (1982), a M.S. in Chemical Engineering (1985) and Ph.D. in Chemical Engineering (1990) all from Texas A&M University. I have worked in the field of and on the product and process development efforts related to barley beta glucan technology for 10 years.
2. I have read and understood U.S. Patent Application No. 10/817,643 to Zheng et al. (the Cargill Application).
3. I have read and understood U.S. Patent No. 7,138,519 to Morgan (Reference A).
4. I have read and understood the following documents:
    a. "Rapid Communication – Glucagel™, A Gelling β-Glucan from Barley," Morgan, Keith R. Ofman, Diana J., Journal of Cereal Chemistry, Vol. 75, No. 6, pp 879-881, (1998) (Reference 1).
    b. U.S. Patent Application No. 10/167,613 to Morgan (Reference 2).
    c. "Randomized Controlled Crossover Study of the Effect of a Highly β-glucan-enriched Barley on Cardiovascular Disease Risk Factors in Mildly Hypercholesterolemic Men," Keogh, Geraldine F. et al, American Journal of Clinical Nutrition, Vol. 78; pp 711-8, (2003) (Reference 3).
    d. "The Effects of Concentrated Barley β-glucan on Blood Lipids in a Population of Hypercholesterolaemic Men and Women," Keenan, et al., British Journal of Nutrition, pp 1-7, (2007) (Reference 4).
    e. Glucagel™ Product Description and Characterization Sheet (Reference 5).
5. I have been asked to comment on the product produced by the process of the Cargill Application and whether that product would inevitably differ physically and chemically from the products produced by the processes of Reference A.
6. The products produced by the processes of Reference A are consistently less than 80 kDa and this low molecular weight (MW) results in a documented lack of health benefits of Keith R. Morgan's product, as reported in Reference 3.
7. In Reference 1, the authors describe making a barley beta-glucan product they call "Glucagel™." As shown by Reference 5, the MW of Glucagel™ is between 50-60 kDa. One of the key features of the process for making Glucagel™ is allowing the native enzymes in the barley to hydrolysis beta-glucans. They state:

    > "First, there is no enzyme deactivation step; in fact, enzyme hydrolysis of the cell wall β-glucan and the solubilized β-glucan that is formed, is an integral and necessary part of the process." Page 880, column 1, paragraph 3.

8. This feature results in the decreased MW of the barley beta-glucan as further stated in Reference 1:

"Whatever may be the action of the enzymes, enzyme hydrolysis occurs during extraction and results in a *decrease in the molecular weight of the Glucagel™* with increasing extraction time." Page 880, column 1, paragraph 5. Emphasis added.

9. These statements would be consistent with Table 1 in Reference 1, page 880, where the MW of ten of the 11 examples reported in the table are less than 80 kDa.

TABLE I
Glucagel Yields as a Percentage of the Weight of the Flour,[a] Percentage of β-Glucan Content, and Molecular Weights[b]

Extraction Temperature (°C)	Extraction Time (hr)	Glucagel Yield (%)	β-Glucan Content (%)	Molecular Weight at Peak Apex[c]
25	0.5	1.8	···	62,000
25	2	1.9	94	53,000
25	3.5	1.6	92	46,000
25	5	1.6	···	37,000
35	5	1.6	···	30,000
40	0.5	2.5	82	79,000
45	2	2.8	90	49,000
45	5	1.9	91	31,000
55	2.75	4.7	90	62,000
55	5	3.6	94	56,000
55[d]	0.5	2.7	89	560,000

[a] Milled barley pollard flour produced from a breeders selection containing 6.8% β-glucan.
[b] With respect to pullulan standards.
[c] In gel-permeation chromatography.
[c] Pollard flour from the bran finisher.

10. There is a typo in the table where the second "c" footnote should be "d".
11. The molecular weight reported for "d" (560 Daltons) is clearly an outlier compared to the other results and can be explained as follows.
12. The type of flour used in the examples reported in Table 1, Reference 1 is milled barley pollard flour.
13. The structure of barley grain contributes to the very high molecular weight value of the pollard flour from the bran finisher, especially as compared to the other samples. Like all grains, after the hull is removed from the grain, there is the outer coat of the grain called the bran. The two major internal structures of the grain are the endosperm (where starch is stored) and the germ (portion of the grain which grows into a new plant). In barley, the majority of native enzymes (amylases, beta-glucanases, etc.) are stored or synthesized during malting in the bran layer. Beta-glucan in barley is stored in the endosperm portion of the grain via the cell walls holding the starch granules in place, and along with the bran layer. In contrast, the majority of beta-glucan in oats, for example, is stored in the bran layer, not in the endosperm.
14. Barley is similar to wheat in milling into flour. The goal of milling grains is recovery the starch for flour use, i.e. removal of the starch from the grain components – bran, germ, and cell wall. The first major step in the milling

process is a series of "breaks" which fracture the grain into large particles. Depending on the equipment/facility there can be a series of breaks to reduce the size of the particles. With each break, the particles are sifted to remove the starch from the particles. The flour from the break section of the milling process is considered the highest quality because of the least amount of bran present in the flour. The large bran particles are eventually cleaned with bran finishers which are devices which remove the endosperm (starch and cell wall) material from the bran before discharge. This would be the pollard flour from the bran finisher.

15. After the break section in the milling process, there is what is called the reduction section where material which was not recovered in the break section (flour and large bran particles), is further reduced in size and sifted to recover the starch granules. The bran and large cell wall material continue to be milled and sifted to recover as much starch as possible. Processing through the reduction section results in flour as well as the bran/cell wall material which is referred to as shorts or pollard flour. This material will be high in beta-glucan but will also contain bran fragments which lead to the results reported by Morgan.
16. Morgan tested the pollard fraction from the bran finisher, which had the least amount of bran and thus the least amount of native enzymes from the barley. The MW value of 560,000 daltons occurred because there *were no native enzymes* present during the sample processing. Morgan isolated native beta-glucan, not a material which was hydrolysis.
17. With respect to the other results reported in Table 1, the material included barley pollard flour (page 879, column 2, paragraph 4), which has bran fragments and therefore native enzymes to hydrolyze the beta-glucan as supported by the results.
18. In Reference 2, Keith R. Morgan describes a "process of obtaining β-glucan from cereal by extracting with water and without deactiviation of enzymes associated with the cereal." In example 8, Table 1 from Reference 1 is reproduced almost verbatim as Table 2 (page 5, column 1, paragraph 0073). The MW values reported in both tables are reproduced below to illustrate the similarity.

Extraction Temperature (°C)	Extraction time (hr)	MW from Table 1, Reference 1	MW from Table 2, Reference 2
25	0.5	62,000	62,000
25	2	53,000	53,000
25	3.5	46,000	45,000
25	5	37,000	36,000
35	5	30,000	29,000
40	0.5	79,000	79,000
45	2	49,000	49,000
45	5	31,000	31,000
55	0.5	560,000	> 100,000
55	2.75	62,000	62,000
55	5	56,000	35,000

19. Again, as in Reference 1, Keith R. Morgan reports MW values where 10 out of 11 are less than 80 kDa. However, in Reference 2, the distinction made for

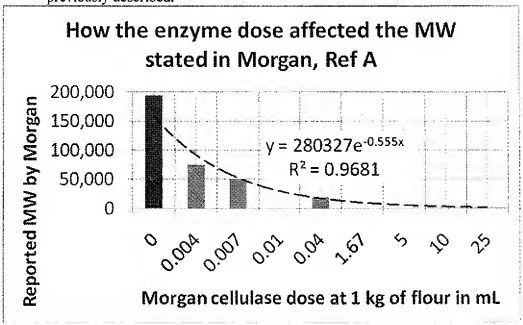
the starting material for the 55°C, 0.5 hour extraction is not reported. In example 5, page 4, column 1, paragraph 0069 of Reference 2, Keith R. Morgan reports at 55°C and ½ hour extraction with "finished pollard barley flour fraction," the beta-glucan has a MW of 560,000 daltons, like in Reference 1. In example 8, page 4, column 2, paragraph 0072, Keith R. Morgan states that a series of experiments with "barley pollard fraction" was extracted. Thus, while one cannot say with certainty which pollard flour Keith R. Morgan is referring to, one of skill in the art would conclude that the pollard flour had the least amount of bran and thus the least amount of native enzymes from the barley. Accordingly, the MW value of >100,000 daltons occurred because there *were no native enzymes* present during the sample processing and native beta-glucan was isolated, not a material which was hydrolysis.

20. Likewise, in Reference A, one can see again that Keith R. Morgan is generating MW's of less than 80 kDa. In Reference A, rather than relying on native enzymes in the barley, Morgan describes treating a mixture of water and barley flour with cellulase to reduce the MW of the beta-glucan (page 2, paragraph 0026; examples 2, 3, 5, 6, 8, 9, 10, 14, 17, and 19; and claims 12 and 17).
21. From the information revealed in Reference A, one can construct the following table of the ratio of enzyme (e.g. cellulase) to flour and the reported product MW, where in Example 10 Keith R. Morgan *does not* use cellulase enzyme to modify the MW of the beta-glucan. This is the native/original MW of the barley, not a modified MW product of the process reported in Reference A.

Example	Page	Col	Paragraph	Ex ratio	1 kg	MW
2	4	1	59	1 gm : 10 µL	1 kg : 10 mL	Not Reported (nr)
3	4	1	61	2 gm : 10 µL	1 kg : 5 mL	nr
5	4	2	63	30 gm : 50 µL	1 kg : 1.67 mL	nr
6	5	1	68	30 gm : 50 µL	1 kg : 1.67 mL	nr
8	5	1	71	25 gm : 125 µL	1 kg : 5 mL	nr
9	5	2	72	0.2 gm : 5 µL	1 kg : 25 mL	nr
10	5	2	73	5 gm : 0.2 µL 5 gm : 0.02 µL 5 mg : 0 µL	1 kg : 0.04 mL 1 kg : 0.004 mL 1 kg : 0 mL	19,000 75,000 194,000
14	6	1	79	5 gm : 0.05 µL	1 kg : 0.01 mL	nr
17	6	2	83	10 gm : 0.1 µL	1 kg : 0.01 mL	nr
19	7	1	86 & 87	69 kg : 0.5 mL	1 kg : 0.007 mL	~50,000

22. The data from the above table is further illustrated by the following Figure where the left most bar is the MW for native beta glucan material. As shown by the figure, and as one skilled in the art would realize, enzyme doses *greater* than 0.004 ml/1 kg of flour, will result in a MW *less* than 75 kDa. Thus even though Reference A does not report the MW for all of the examples described in Reference A, and as reported in the table above, one of skill in the art would recognize that the process of Reference A would not result in a modified beta glucan material with MW greater than 75 kDa. This is consistent with the

results and data reported by Keith R. Morgan in References 1 and 2 as previously described.



23. Although Reference A does not use the term "Glucagel™", the word "gel" or "gelling" appears 59 times in the text, and in Claims 1, 22, 23, 24, 25, 26, 27, 32, 34, 36, and 38. Someone skilled in the art would conclude that Morgan in Reference A is describing Glucagel™ production with added enzymes versus without enzymes as described in Reference 1 and 2.
24. Though Morgan does not make claims about uses of Glucagel™ in Reference 1, in Reference A, Keith R. Morgan makes the following claims:

    1) the product can lower serum cholesterol levels (Page 2, Col 1, paragraph 0030; and page 8, col 2, claim 42); and
    2) the product can moderate glycaemic response (page 2, col 1, paragraph 0032; and page 8, col 2, claim 44).

25. Reference 3 is an article from the American Journal of Clinical Nutrition, Keogh reporting results from a study about the efficacy of a highly beta-glucan-enriched barley on cardiovascular disease risk factors in mildly hypercholesterol men. In this article, Glucagel™ was the barley beta-glucan product tested. The article states that:

    "The barley β-glucan fed in this trial was given as a highly enriched barley fiber product, a gelling form of β-glucan (Glucagel™; Graceline Ltd, Christchurch, New Zealand), produced from high β-glucan content barley that was milled and sieved to separate the starch and cell-wall material. A 2-step extraction process was carried out to produce the β-glucan–enriched product: *1*) water extraction at 50–60 °C and *2*) a freeze-and-thaw extraction from which the β-glucan was recovered. The final product was a (1→3)(1→4)-β-D-glucan (β-glucan), comprising 75% (by wt) β-glucan that was insoluble in cold

water and that formed a weak gelling agent." Page 712, Section – Treatment, 1st paragraph

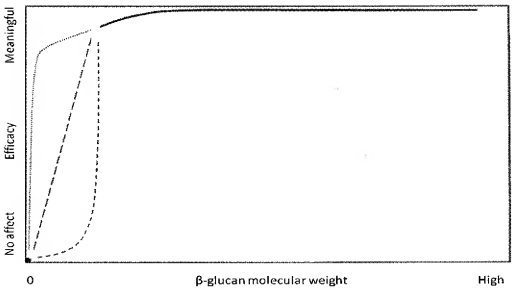
26. The study found that Glucagel™ was not effective in either of the two areas claimed by Morgan:

> "In conclusion, this current trial—which investigated the efficacy of a highly β-glucan–enriched barley product—*did not show clinically significant improvements in lipid or glucose control*, and thus there was no evidence of improvement in CVD or type 2 diabetes risk in this group of young to middle-aged, mildly hypercholesterolemic men. Because β-glucan was previously shown to be highly efficacious at doses as low as 3 g/d, we suggest that this lack of effect may be, at least in part, a *consequence of structural changes in β-glucan* that result from the commercial processing of the barley into a highly enriched β-glucan product or from the freezing, storage, or baking of the product during the intervention period." Emphasis added. Page 716, Column 2, last paragraph.

27. Cargill has tested its modified MW beta-glucan product of the Cargill Application to determine its efficacy in affecting cardiovascular disease marks. The study and results as reported in Reference 4, revealed a decrease in health benefits as the MW of the beta-glucan product is decreased. The MW of the High MW product was determined to be ~1,000 kDa, while the Low MW product was determined to be 150 ± 20 kDa.

Dose	5 grams / day		3 grams / day	
Molecular weight	High	Low	High	Low
Total Cholesterol	-12%	-11%	-8%	-7%
LDL – C	-15%	-13%	-9%	-9%

28. Thus, according to References 3 and 4, Glucagel™ is not efficacious in affecting cardiovascular disease markers but the beta-glucan product of the Cargill Application is. It is my contention that the MW of the product is the key factor. This trend would be consistent with the idea that if beta-glucan were fully hydrolysis, that is, reduced to just glucose molecules, there would be no health benefits as compared to oat and barley products. This trend can be depicted by the following Figure which illustrates how the native MW beta-glucans found in oats and barley provide the observed health benefits, but a very reduced MW beta-glucan (glucose) will not. Where the break point on the relationship between Efficacy and MW is has yet to be definitively determined, as shown by the three broken lines to illustrate the unknown relationship.



29. Through my work on the product and process development efforts related to barley beta glucan technology, it has been learned that the health benefits decrease as the MW is decreased. If the products reported by Keith R. Morgan were in the less than 80 kDa range (and References A, 1 and 2 would support this assumption at the time of his work), this would explain why the products reported by Keith R. Morgan product has not been found to be efficacious according to Reference 3 even though the doses of the Glucagel™ product were 8.1 to 11.9 grams of β-glucan / day, significantly higher than the doses reported in Reference 4 using the product of the Cargill Application.
30. Thus, while both Reference A and the Cargill Application report a modified MW beta-glucan, the Cargill Application provides a product with a MW range where the product is still efficacious in lowering serum cholesterol in human subjects. Reference A teaches either how to make a β-glucan product with a MW below the 80 kDa value having with a unique gelling characteristic or high MW beta-glucans (defined at page 2, col 2, paragraph 0038 has having a MW greater than 500 kDa) that can lower serum cholesterol levels (page 2, col 2, paragraph 0040). Reference A does not disclose a low MW beta-glucan product barley that can provide the claimed health benefits.
31. In stark contrast, the Cargill Application controls the MW of the barley beta-glucan product to the range of 120 to 400 kDa to maintain the health benefits.

/Dr. Richard Hess/ May 19, 2010

Dr. Richard Hess Date